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Pharmacokinetics of the protein microbicide 5P12-RANTES in sheep following single dose vaginal gel administration

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Abstract

5P12-RANTES, a chemokine analogue that potently blocks the HIV CCR5 co-receptor, is being developed as both a vaginal and rectal microbicide for prevention of sexual transmission of HIV. Here, we report the first pharmacokinetic data for 5P12-RANTES, following single dose vaginal gel administration in sheep. Aqueous gel formulations containing low (1.24 mg/mL), intermediate (6.16 mg/mL) and high (32.0 mg/mL; suspension-type gel) concentrations of 5P12-RANTES were assessed via rheology, syringeability and *in vitro* release testing. Following vaginal gel administration in sheep, 5P12-RANTES concentrations were measured in vaginal fluid, vaginal tissue and serum over a 96-h period. All gels showed non-Newtonian pseudoplastic behaviour, with high concentration gels exhibiting greater viscosity and cohesive structure. In *in vitro* release testing, >90% 5P12-RANTES was released from the low and intermediate gels after 72 h. For the high concentration gel, ~50% 5P12-RANTES was detected, attributed to protein denaturation during lyophilisation and/or subsequent solvation of the protein within the gel matrix. In sheep, 5P12-RANTES concentrations in vaginal fluid, tissue and serum increased in a dose dependent manner. Highest concentrations were measured in fluid (10^5–10^7 ng/mL) followed by tissue (10^4–10^6 ng/mL) – both several orders of magnitude above reported half maximal inhibitory concentrations – and lowest in serum (< 10^2 ng/mL). The 5P12-RANTES pharmacokinetic data is similar to that reported previously for other candidate microbicides. These data, coupled with 5P12-RANTES's picomolar potency, its strong barrier to resistance, and full protection observed in a rhesus macaque vaginal challenge model, support the continued development of 5P12-RANTES as a microbicide.

Introduction

In the continued absence of an effective vaccine against HIV, development of vaginally-administered microbicide products remains a priority biomedical strategy aimed at addressing the urgent and yet unmet need for reducing women's risk of sexually-transmitted infection with HIV, particularly in developing countries (1, 2). Small-molecule antiretrovirals – most notably dapivirine (3–10), tenofovir (11–15) and tenofovir disoproxil fumarate (14, 16–19) – are currently the primary focus for the microbicide field, offering relatively low costs for synthesis of the active pharmaceutical ingredient, a diverse range of practical formulation options, and potent antiviral activity targeted at the reverse transcription step in the HIV replication cycle. However, there is a strong rationale for the development of new antiviral agents for HIV prevention that overcome some of the disadvantages associated with these lead candidate, small molecule, antiretroviral microbicides, including the potential emergence of resistant viruses and their post-entry mechanism of action (20–22). By comparison, many of the larger molecular weight biopharmaceutical compounds that have been or are being considered as vaginal microbicides – including cyanovirin-N (23–25), Griffithsin (26, 27), 5P12-RANTES (28, 29), T-1249 peptide (30), retrocyclin RC-101 (31) and monoclonal antibodies (32–34) – generally exhibit greater antiviral potency than current small molecule antiretrovirals (at least in *in vitro* models) and usually act prior to viral entry into the host cell by either directly targeting the free virus or by blocking cell receptors (35–38).

PSC-RANTES is a highly potent CCR5-inhibiting protein and an analogue of the natural CCR5 chemokine ligand RANTES (39). PSC-RANTES interaction with the CCR5 receptor leads to

intracellular sequestration of the receptor and prevention of HIV binding and infection (40). Although PSC-RANTES has previously shown full protection in a macaque challenge study (41), it requires expensive chemical synthesis (28). 5P12-RANTES, by comparison, is a fully recombinant analogue of PSC-RANTES that was first identified using a modified phage display selection strategy (42). Similar to PSC-RANTES, 5P12-RANTES exhibits picomolar anti-HIV potency and afforded complete protection against SHIV infection in a rhesus vaginal challenge study (29, 42). Notably, in that challenge study, 5P12-RANTES was administered vaginally in a PBS solution 30 min prior to simian human immunodeficiency virus (SHIV) exposure. Clinical grade 5P12-RANTES can be produced to cGMP standards using low-cost production through industrial microbial fermentation methods commonly employed for proteins used in the food and detergent industries (42, 43). Furthermore, this analogue inhibits the CCR5 receptor without inducting receptor internalization or signal activation (42). This is particularly important from a clinical perspective as CCR5 activation can induce inflammation, a risk factor linked to enhanced susceptibility to HIV infection (44).

As part of our ongoing efforts towards developing sustained release vaginal formulations for 5P12-RANTES (including new vaginal ring strategies), we report here for the first time the pharmacokinetics of vaginally-administered 5P12-RANTES measured following single dose aqueous gel administration in the sheep model. The study is the first to report pharmacokinetic data for 5P12-RANTES.

Results

Visual appearance of gels

The low and intermediate concentration 5P12-RANTES gels were clear viscous gels, similar in visual appearance and viscosity to the placebo microbicide gel. The high concentration gel, in which 5P12-RANTES was deliberately incorporated above its solubility limit, was white in appearance due to the presence of suspended lyophilised 5P12-RANTES material.

Rheological and syringeability assessment of 5P12-RANTES gels

Rheograms of viscosity vs. shear rate are presented in Fig. 1A, and indicate non-Newtonian shear thinning pseudoplastic behaviour, meaning that the viscosity decreases with increasing shear rate. Apparent gel viscosity, as measured by application of the Power Law, also generally increased with increasing 5P12-RANTES concentration (Figure 1B). Significantly higher viscosity values were measured for high dose gels compared to blank, low and medium dose gels, attributed to the presence of both solid (i.e. dispersed lyophilisate) and dissolved 5P12-RANTES in the high dose gel and only dissolved 5P12-RANTES in the low and intermediate gels. Intermediate and low gels had a significantly greater viscosity than the blank gel, attributed to the pH of the 5P12-RANTES stock solution (6.4 mg/mL in 1.7 mM acetic acid; pH 4.0) weakly affecting the HEC gel.

Syringeability is a measure of the work required to expel a liquid-based or semi-solid formulation (including vaginal gels) from an applicator or through a syringe needle (Figure 1C) (45–50).

Syringeability values ranged from 107–130 N.mm and increased with 5P12-RANTES loading, although differences were mostly not significant (Figure 1D).

In vitro release of 5P12-RANTES

The *in vitro* cumulative release vs. time plots for 5P12-RANTES gel formulations into SVF are presented in Figure 2 and reveal the effect of initial 5P12-RANTES loading on drug release. The low and intermediate gels provided release of >90% of the original 5P12-RANTES loading after 72 h (4.6 and 32.6 mg, respectively). However, the high concentration gel provided only 50% release (64.4 mg), despite there being no gel residue visible (or dispersed 5P12-RANTES lyophilisate material) at the end of the release experiment.

Sheep pharmacokinetics

The three HEC gel formulations containing 5P12-RANTES showed no detrimental effects on the health of the animals. 5P12-RANTES was measured in all post-dose vaginal fluid samples (Figure 3a), with mean values steadily declining from a high of 10^6–10^7 ng/g 1 h after dosing to 10^2–10^4 ng/g 96 h after dosing. For most sampling timepoints, vaginal fluid concentrations of 5P12-RANTES increased with increasing 5P12-RANTES loading in the gel. (It should be noted that Weck-Cel vaginal sampling cannot differentiate between sampling of any gel resident in the vagina and sampling of the fluid itself.)

5P12-RANTES was also measured in all post-dose vaginal biopsy homogenate samples (Figure 3b); 12 h after gel dosing, mean 5P12-RANTES concentrations values in tissue were 1.75×10^5,

125 3.53×10^5 and 1.03×10^6 ng/g for the low, intermediate and high gels, respectively. At 96 h, mean
126 tissue levels for the high concentration gel (the only gel formulation for which tissue biopsies
127 were taken at this timepoint) had declined to 9.2×10^3 ng/g.
128
129 5P12 RANTES was detected in some, but not all, serum samples, at concentrations <70 ng/mL.
130 Of the 36 serum samples taken for each gel formulation (4 sheep x 9 timepoints), 18, 13 and 4
131 samples in the low, intermediate and high gel groups, respectively, had 5P12-RANTES
132 concentrations below the lower limit of quantification (0.176 ng/mL). Plots of mean (n=4) 5P12-
133 RANTES concentration vs. time are presented in Figure 3c for the three gel formulations. For
134 the most part, the profiles are similar, showing peak concentrations between 3 and 8 h after
135 dosing and subsequently declining to ~0.2 ng/mL by the 96 h timepoint (Table 1). The serum
136 concentrations measured for the intermediate and high concentration gels are similar, despite
137 the higher 5P12-RANTES loading in the high concentration gel.
138
139 Based on these pharmacokinetic profiles, the key pharmacokinetic parameters – C_{max}, t_{max} and
140 *AUC* – are reported in Table 1. The C_{max} and *AUC* values in both serum and vaginal fluid increase
141 with increasing dose. The relatively small and non-linear increase observed on comparing the
142 high dose gel with the intermediate dose gel is due to the presence of solid 5P12-RANTES in the
143 high dose gel. Serum t_{max} values for 5P12-RANTES, although variable, ranged from 1–96 h, and
144 most commonly 4 h. In vaginal fluid, t_{max} values were also variable but mostly in the range 1 to
145 12 h post dosing. Finally, individual sheep PK profiles are presented in Figure 4.
146

Discussion

In this study, vaginally-administered 5P12-RANTES was measured following single dose aqueous gel administration in sheep. The pseudoplastic behaviour of the gel, and indeed the general magnitude of the viscosity values, is typical of gels intended for vaginal drug administration, as reported previously for aqueous hydroxyethylcellulose gels and non-aqueous silicone gels (50–57). From a clinical perspective, pseudoplasticity offers certain advantages and disadvantages. Gels will likely spread more easily at higher shear rates (during coitus) or following dilution with vaginal fluids or semen leading to increased distribution in the vaginal environment and enhanced tissue coverage (51). However, there is also an increased likelihood of leakage of the gel from the vagina (58–60). The syringeability values are higher than values reported previously for other water-based vaginal gels (e.g. 35–67 N.mm for rheologically structured vehicles (46, 49), and 4.4 N.mm for Replens™ vaginal moisturiser (49)), although the test method used in this study involved testing expulsion of a greater quantity of gel (4 g vs. 3 g) through a greater plunger distance (50 mm vs. 30 mm) and the use of a commercial plastic vaginal applicator rather than a general purpose disposable plastic syringe. All things considered, the syringeability values measured translate into ease and practicality of gel product administration, as verified by the animal handlers.

In the *in vitro* release testing study, a relatively low release/recovery value (~50%) for 5P12-RANTES was noted for the high dose gel, compared to >90% release from the low and intermediate gels. We attribute this low recovery to the rendering of the 5P12-RANTES inactive

following the lyophilisation process and/or the subsequent dissolution of the lyophilisate in the *in vitro* release medium.

5P12-RANTES has previously been shown to be stable by *in vitro* cell fusion assay (i) when exposed to elevated temperatures(up to 55°C in water for 24 h and 40°C for 1 week), (ii) at normal vaginal pH for 24 h, (iii) and when incubated with human cervicovaginal lavage or human semen at 37°C for 24 h (39). The data presented here suggest for the first time that 5P12-RANTES is also sufficiently stable *in vivo* to allow detection and measurement of the protein by Weck-Cel sampling of sheep vaginal fluid, as reported previously for various small molecule HIV microbicides (61–64). However, as previously noted for the *in vitro* fusion assay (39), protein modifications that may affect 5P12-RANTES activity can not be completely excluded due to using ELISA and more appropriate analytical techniques will be required when undertaking further preclinical development. Only a small number of studies describing vaginal delivery of peptides or proteins, and particularly systemic absorption, are reported in the literature (65–69).

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5P12-RANTES has previously been shown to fully protect against SHIV infection in a rhesus macaque vaginal challenge model following vaginal administration of 4 mL of a 1 mmol/L 5P12-RANTES solution in PBS (29). For reference, 1 mmol/L = 7.90 mg/mL based on a molecular weight of 7904.8 g/mol for 5P12-RANTES, and therefore the overall dose administered in the macaque challenge study was 31.6 mg (compared to the 24.7 mg dose administered in this study using the intermediate concentration gel). In that challenge study, inoculation with 300 $TCID_{50}$ SHIV162P3 occurred 30 min after gel dosing. Based on our pharmacokinetic data in

sheep which used the same dosing volume of 5P12-RANTES gel, and assuming similar distribution and drug absorption characteristics between the species, we estimate 5P12-RANTES concentrations in the protected macaques at the time of challenge of ~10^7 ng/g in vaginal fluid, ~10^5 ng/g in vaginal tissue, and ~ 1 ng/mL in serum.

The 5P12-RANTES concentrations measured here in the three biological compartments in sheep are, in fact, similar in magnitude to those measured for other microbicide candidates administered to women by vaginal gel (60, 70–72). Clearly, the inclusion of additional dispersed 5P12-RANTES in the high concentration gel beyond the solubility limit achieved with the intermediate gel does not drastically contribute to increased absorption of 5P12-RANTES. However, the high dose formulation might give rise to sustained release due to a depot-type effect following vaginal administration of the gel. The PK data does seem to support the hypothesis that lyophilized 5P12-RANTES contained within the gel leads to extended pharmacokinetic exposure as indicated with vaginal fluid at the 72 and 96 h time points, despite the limited recovery of 5P12-RANTES from the lyophilized material *in vitro*. Further analysis reveals that the t_{max} values are greater compared with those usually measured for small molecule antiretrovirals, such as maraviroc (73), and indicate that 5P12-RANTES is less well absorbed by the systemic compartment. This is not surprising given the relatively large molecular weight and greater hydrophilic character of 5P12-RANTES. Overall, these serum data demonstrate that solubilised 5P12-RANTES within the gel formulations is capable of being absorbed into the systemic compartment and that serum concentrations are significantly lower than vaginal fluid and tissue levels, as is preferred for any locally acting HIV microbicide.

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Following previous data demonstrating that vaginal administration of 5P12-RANTES offered full protection in a macaque vaginal challenge model (29) and that 5P12-RANTES is biologically stable (39, 74), the 5P12-RANTES pharmacokinetic data presented here in sheep is encouraging and supports continued development of 5P12-RANTES as a low-cost, stable, highly potent, HIV microbicide candidate.

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Materials and Methods

Ethics

The study was carried out in compliance with applicable sections of the United Kingdom Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012. The study complied with the applicable sections of the Codes of Practice for the Housing and Care of Animals used in Scientific Procedures, and the Humane Killing of Animals under Schedule 1 of the Act.

Materials

A solution of recombinant 5P12-RANTES, 7.9 kDa, (6.4 mg/mL in 1.7 mM acetic acid; pH 4.0) produced as described previously(43) was supplied to Queen's University Belfast (Belfast, UK) by collaborators at the Mintaka Foundation for Medical Research (Geneva, Switzerland). Pharmaceutical grade hydroxyethylcellulose (Natrosol® 250HX Pharm; HEC) and glycerol were obtained from Ashland UK Limited (Manchester, UK). Sorbic acid (preservative), sodium chloride, potassium hydroxide, calcium hydroxide, lactic acid, acetic acid, glucose, and bovine serum albumin (BSA) (most for preparation of simulated vaginal fluid) were purchased from Sigma-Aldrich (Gillingham, UK) (75). Urea was obtained from VWR International (UK). Polyethylene vaginal applicators (pre-filled design; capped; volume 5.5 cm^3) were purchased from HTI Plastics (Lincoln, Nebraska, USA).

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Preparation of 5P12-RANTES loaded HEC gels

The composition of the 5P12-RANTES gel formulations was based on the Universal Placebo gel that has previously been developed as a control gel for clinical testing of vaginal microbicides [+

3 REFS]. Separate gel batches were prepared for *in vitro* and pharmacokinetic testing. For each batch of intermediate concentration 5P12-RANTES gel (6.18 mg/mL), 0.1% w/w sorbic acid (0.0250 g), 0.85% w/w sodium chloride (0.2125 g), and 2.7% w/w HEC (0.6750 g) were added to 25 mL of the 5P12-RANTES stock solution and then mixed in a SpeedMixer™ (2 x 30 s at 3000 rpm; DAC 150 FVZ-K, Hauschild, Germany). The resulting gel was allowed to fully hydrate overnight at 4–6°C. For the low concentration gel (1.24 mg/mL), the stock 5P12-RANTES solution was first diluted 1:4 with Type 1 water (Millipore Direct-Q 3 UV Ultrapure Water System, Watford, UK), before addition of 0.1% w/w sorbic acid, 0.85% w/w sodium chloride, and 2.7% w/w HEC. For manufacture of the high concentration gel, 0.1% w/w BSA was first added to the stock 5P12-RANTES solution and 20 mL aliquots of the resulting solution lyophilised (AdVantage Pro BenchTop Freeze Dryer, VirTis. Gardiner, NY, USA) in individual petri dishes of known weight. The freeze-drying procedure was as follows; material was frozen by cooling from 5 to −40°C over 1 h, with additional freeze of 2 h. The condenser was set to -50°C with initial chamber pressure of 50 mTorr. The shelf was then raised to 20°C over 27 h with simultaneous increase in chamber pressure as required. The required weight of the resulting lyophilisate was added slowly with gently with gentle manual mixing to ultrapure Type 1 water containing 0.1% w/w sorbic acid, 0.85% sodium chloride, and 2.7% w/w HEC, speedmixed (2 x 30 s at 3000 rpm) and hydrated overnight at 4–6°C to produce the final gel formulation with a 5P12-RANTES concentration of 32.0 mg/mL. Gel formulations were adjusted to pH 4.5 ± 0.2 and stored at 4–6°C immediately after preparation.

Gels were filled into single-use, disposable, screw-top, plastic vaginal applicators. Each gel was mixed (SpeedMixer™; 30 s, 3000 rpm), transferred to a syringe, and 4.00 g gel was slowly and carefully dispensed into each applicator to avoid incorporation of air bubbles. Applicators were then sealed with the screw top. For the sheep study gels were shipped to the animal testing facility under controlled temperature conditions (2–8°C).

Rheological and syringeability assessment of gels

The rheological behaviour of the gels was assessed by continuous flow rheometry using an AR 2000 Rheometer fitted with a 40-mm diameter stainless steel parallel plate (TA Instruments, USA). Gel samples were applied to the base plate and the steel parallel plate was lowered to a gap distance of 1000 µm. Excess gel was removed before the test. Testing was performed at 37°C in continuous ramp mode from 0.01–100 s^{-1}. Gel viscosity was determined using the Power Law applied to the linear portion of the resulting log-log plot of viscosity vs. shear rate.

Gel syringeability was assessed by measuring the work required to expel 4.0 g of 5P12-RANTES-loaded HEC gels from plastic vaginal applicators using a Texture Analyser (TA-XT2, Stable Microsystems, UK) fitted with a texture profile analysis (TPA) probe. Applicators were secured vertically and the probe was lowered to 5.0 mm above the barrel plunger. In compression mode, the probe was programmed to move at a rate of 2.0 mm/s through 50 mm upon contact with the plunger (1.0 mm/s before contact, with a trigger force of 0.025 N). The work was calculated by measuring the area under the resultant force-distance plot.

In vitro dissolution testing of 5P12-RANTES gels

Simulated vaginal fluid (SVF), modified to include an increased BSA concentration (0.1% w/w) to reduce adsorption of 5P12-RANTES to glassware, was prepared according to previously described methods (75, 76). The SVF was filtered immediately after preparation (sterile Millex-GS Syringe Filter Unit, mixed cellulose ester membrane, Millipore, UK) and stored for a maximum of 3 days at 4–6°C prior to use.

Each gel (4.0 g) was syringed into a sealed glass flask containing SVF (25 mL) and stored in a shaking orbital incubator (Infors HT AGCH-4103; 37°C, 60 rpm, 25 mm throw). A 1.0 mL sample was taken periodically and replaced with an equivalent volume of fresh SVF. Samples were carefully taken from the top of the SVF volume to avoid disturbing or sampling any residual gel sample. Samples were subsequently stored at −20°C prior to analysis by enzyme-linked immunosorbent assay (ELISA).

The concentration of 5P12-RANTES in the SVF release medium was measured by ELISA using the Human CCL5/RANTES ELISA kit (R&D Systems, UK, cat no. DRN00B). Samples were analysed per the manufacturer's instructions after appropriate dilutions were made to ensure concentrations fell within the 0.002–2 ng/mL range of the kit. Absorbance was measured at 450 nm using a microplate reader (Enspire Multimode Plate Reader, PerkinElmer, USA) and the optical density at 570 nm was subtracted to correct for plate imperfections. The standard curve was transformed to a four-parameter logistic (4-PL) curve-fit and sample concentrations were calculated relative to it. Concentration readings were subsequently multiplied by the appropriate dilution factor.

Sheep pharmacokinetic study

Pharmacokinetic evaluation of the three 5P12-RANTES gel formulations following single dose vaginal administration was conducted at Envigo (Huntingdon, UK) using approximately 3-year old Welsh Mule ewes as the test species. Sheep were housed in indoor pens with wheat straw bedding. The animals were provided with natural light, supplemented with overhead fluorescent lighting as necessary, and full fresh air. The animals grazed on ewe and lamb pencil pelleted diet, with good quality meadow hay *ad libitum* and unrestricted water supply. A total of four sheep were used in the study. Initially, each sheep received a single dose of the low concentration gel, followed by periodic sample collection over a 96-h period, and a subsequent 7-day wash out. The intermediate and high concentration gels were then tested according to the same general schedule. Each animal was restrained in a comfortable standing position on the day of gel administration and a 4.0 g dose administered using a pre-filled plastic vaginal applicator. Clinical condition, body weight, and food consumption were recorded during the study. Blood and vaginal fluid was sampled at multiple time points during all three phases. Blood samples were drawn from the jugular vein and incubated for a minimum of 60 min at room temperature to facilitate clotting. Subsequently, the samples were centrifuged (1500 g, 15 min) to separate serum, and then divided and stored at −20°C in polypropylene tubes. Vaginal fluid was sampled using a pre-weighed Weck-Cel sponge that was again weighed after fluid uptake. The sponge was held in place in the vagina for 1 min and stored at −20°C in borosilicate glass tubes. Vaginal tissue biopsies (5 mm diameter) were sampled from the dorsal aspect of the vagina under local anaesthetic (isoflurane/oxygen used where necessary) once per animal in the low and

intermediate gel experiments (12 h post gel application), and twice in the high concentration gel experiment (12 and 96 h post gel application). Analgesia was administered 30 min prior to vaginal biopsy collection. Samples were rinsed with RPMI 1640 and weighed prior to freezing at −70°C. Frozen samples were submitted for analysis to the Envigo's Department of Biomarkers, Bioanalysis and Clinical Sciences (Immunoassay). Briefly, all tissue samples were homogenised in homogenisation buffer (PBS + 2% Triton X-100 + protease inhibitor cocktail) at 4°C and kept on wet ice at all times. Tissue were placed in a minimum of 3 mL of buffer and the ratio of buffer:sample weight was recorded. Samples were homogenated (GentleMACS Dissociator) and centrifuged at 4, 566 g for 10 min. Supernatant was collected into tubes (Watson labelled PP) and placed on wet ice or stored at –70°C. Analysis was performed using a Human CCL5/RANTES ELISA kit, according to the manufacturer's instructions.

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Transparency declarations

O.H. is the inventor on a patent concerning 5P12-RANTES. It is held by the Mintaka Foundation for Medical Research, a foundation registered in Geneva, Switzerland. O.H. and R.E.O. are

cofounders of the Mintaka Foundation, with the roles of Chief Scientific Officer and Chief Executive Officer, respectively. Other authors have nothing to declare.

Author contributions

R.K.M., J.M., V.K., R.E.O, P.B and O.H conceived the idea for the study and analysis. J.M. manufactured the gels and tested their in vitro performance, under supervision from R.K.M., P.B. and V.K. N.D. and D.C. supervised completion of the sheep pharmacokinetic study. Data analysis was performed by J.M., R.K.M, D.C. and N.D., and the pharmacokinetic study report was drafted by N.D. and D.C., with input from R.K.M. and J.M. R.K.M. and J.M. drafted the manuscript. All authors provided critical reading that further developed the manuscript.

Disclaimer

The views expressed in this publication are those of the authors and not necessarily those of the Wellcome Trust.

References

1. Malcolm RK, Boyd PJ, McCoy CF, Murphy DJ. 2016. Microbicide vaginal rings: Technological challenges and clinical development. Adv Drug Deliv Rev 103:33–56.

2. Antimisiaris SG, Mourtas S. 2015. Recent advances on anti-HIV vaginal delivery systems development. Adv Drug Deliv Rev 92:123–145.

3. Baeten JM, Palanee-Phillips T, Brown ER, Schwartz K, Soto-Torres LE, Govender V, Mgodi NM, Matovu Kiweewa F, Nair G, Mhlanga F, Siva S, Bekker L-G, Jeenarain N, Gaffoor Z, Martinson F, Makanani B, Pather A, Naidoo L, Husnik M, Richardson BA, Parikh UM, Mellors JW, Marzinke MA, Hendrix CW, van der Straten A, Ramjee G, Chirenje ZM, Nakabiito C, Taha TE, Jones J, Mayo A, Scheckter R, Berthiaume J, Livant E, Jacobson C, Ndase P, White R, Patterson K, Germuga D, Galaska B, Bunge K, Singh D, Szydlo DW, Montgomery ET, Mensch BS, Torjesen K, Grossman CI, Chakhtoura N, Nel A, Rosenberg Z, McGowan I, Hillier S. 2016. Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women. N Engl J Med NEJMoa1506110.

4. Nel A, Haazen W, Nuttall J, Romano J, Rosenberg Z, van Niekerk N. 2014. A safety and pharmacokinetic trial assessing delivery of dapivirine from a vaginal ring in healthy women. AIDS 1.

5. Spence P, Nel A, van Niekerk N, Derrick T, Wilder S, Devlin B. 2016. Post-Use Assay of Vaginal Rings (VRs) as a Potential Measure of Clinical Trial Adherence. J Pharm Biomed Anal 125:94–100.

6. Boyd P, Fetherston SM, Mccoy CF, Major I, Murphy DJ, Kumar S, Holt J, Brimer A, Blanda

W, Devlin B, Malcolm RK. 2016. Matrix and reservoir-type multipurpose vaginal rings for controlled release of dapivirine and levonorgestrel. Int J Pharm 511:619–629.

7. Nel A, Smythe S, Young K, Malcolm K, McCoy C, Rosenberg Z, Romano J. 2009. Safety and pharmacokinetics of dapivirine delivery from matrix and reservoir intravaginal rings to HIV-negative women. J Acquir Immune Defic Syndr 51:416–23.

8. Chen BA, Panther L, Marzinke MA, Hendrix CW, Hoesley CJ, van der Straten A, Husnik MJ, Soto-Torres L, Nel A, Johnson S, Richardson-Harman N, Rabe LK, Dezzutti CS. 2015. Phase 1 Safety, Pharmacokinetics, and Pharmacodynamics of Dapivirine and Maraviroc Vaginal Rings: A Double-Blind Randomized Trial. J Acquir Immune Defic Syndr 70:242–9.

9. Murphy DJ, Desjardins D, Dereuddre-Bosquet N, Brochard P, Perrot L, Pruvost A, Le Grand R, Lagatie O, Vanhooren L, Feyaerts M, van Roey J, Malcolm RK. 2014. Pre-clinical development of a combination microbicide vaginal ring containing dapivirine and darunavir. J Antimicrob Chemother 1–12.

10. das Neves J, Martins JP, Sarmento B. 2016. Will dapivirine redeem the promises of anti-HIV microbicides? Overview of product design and clinical testing. Adv Drug Deliv Rev 103:20–32.

11. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, Kharsany ABM, Sibeko S, Mlisana KP, Omar Z, Gengiah TN, Maarschalk S, Arulappan N, Mlotshwa M, Morris L, Taylor D. 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science 329:1168–74.

12. Johnson TJ, Clark MR, Albright TH, Nebeker JS, Tuitupou AL, Clark JT, Fabian J, McCabe RT, Chandra N, Doncel GF, Friend DR, Kiser PF. 2012. A 90-day tenofovir reservoir intravaginal ring for mucosal HIV prophylaxis. Antimicrob Agents Chemother 56:6272–6283.

13. Dobard C, Sharma S, Martin A, Pau C-P, Holder A, Kuklenyik Z, Lipscomb J, Hanson DL, Smith J, Novembre FJ, García-lerma JG, Heneine W, Francis J, García-lerma JG, Heneine W, Pau C-P, Holder A, Kuklenyik Z, Lipscomb J, Hanson DL, Smith J, Novembre FJ, García-lerma JG, Heneine W. 2012. Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. J Virol 86:718–25.

14. Marrazzo JM, Ramjee G, Richardson B a, Gomez K, Mgodi N, Nair G, Palanee T, Nakabiito C, van der Straten A, Noguchi L, Hendrix CW, Dai JY, Ganesh S, Mkhize B, Taljaard M, Parikh UM, Piper J, Mâsse B, Grossman C, Rooney J, Schwartz JL, Watts H, Marzinke M a, Hillier SL, McGowan IM, Chirenje ZM. 2015. Tenofovir-Based Preexposure Prophylaxis for HIV Infection among African Women. N Engl J Med 372:509–518.

15. Rastogi R, Teller RS, Mesquita PMM, Herold BC, Kiser PF. 2013. Osmotic pump tablets for delivery of antiretrovirals to the vaginal mucosa. Antiviral Res 8–11.

16. Srinivasan P, Dinh C, Zhang J, Pau C-P, McNicholl JM, Lo Y, Herold BC, Teller R, Kiser P, Smith JM. 2014. Pharmacokinetic evaluation of tenofovir disoproxil fumarate released from an intravaginal ring in pigtailed macaques after 6 months of continuous use. J Med Primatol 43:364–369.

17. Smith JM, Srinivasan P, Teller RS, Lo Y, Dinh CT, Kiser PF, Herold BC. 2015. Tenofovir disoproxil fumarate intravaginal ring protects high-dose depot medroxyprogesterone acetate-treated macaques from multiple SHIV exposures. J Acquir Immune Defic Syndr 68:1–5.

18. Keller MJ, Mesquita PM, Marzinke MA, Teller R, Espinoza L, Atrio JM, Lo Y, Frank B, Srinivasan S, Fredricks DN, Rabe L, Anderson PL, Hendrix CW, Kiser PF, Herold BC. 2015. A phase 1 randomized placebo-controlled safety and pharmacokinetic trial of a tenofovir disoproxil fumarate vaginal ring. AIDS.

19. Baum MM, Butkyavichene I, Churchman SA, Lopez G, Miller CS, Smith TJ, Moss JA. 2015. An intravaginal ring for the sustained delivery of tenofovir disoproxil fumarate. Int J Pharm 495:579–87.

20. Scott Y, Dezzutti CS. 2015. Non-Antiretroviral Microbicides for HIV Prevention. AIDS Rev 18:145–150.

21. Waheed AA, Tachedjian G. 2016. Why Do We Need New Drug Classes for HIV Treatment and Prevention? Curr Top Med Chem 16:1343–9.

22. Karim QA, Baxter C, Karim SA. 2013. Topical Microbicides — What ' s New ? 63:144–149.

23. O'Keefe BR, Murad AM, Vianna GR, Ramessar K, Saucedo CJ, Wilson J, Buckheit KW, da Cunha NB, Araújo ACG, Lacorte CC, Madeira L, Mcmahon JB, Rech EL. 2015. Engineering soya bean seeds as a scalable platform to produce cyanovirin-N, a non-ARV microbicide against HIV. Plant Biotechnol J 13:884–892.

24. Alexandre KB, Gray ES, Mufhandu H, McMahon JB, Chakauya E, O'Keefe BR, Chikwamba

R, Morris L. 2012. The lectins griffithsin, cyanovirin-N and scytovirin inhibit HIV-1 binding to the DC-SIGN receptor and transfer to CD4+ cells. Virology 423:175–186.

25. Colleluori DM, Tien D, Kang F, Pagliei T, Kuss R, McCormick T, Watson K, McFadden K, Chaiken I, Buckheit RW, Romano JW. 2005. Expression, purification, and characterization of recombinant cyanovirin-N for vaginal anti-HIV microbicide development. Protein Expr Purif 39:229–236.

26. Moulaei T, Alexandre KB, Shenoy SR, Meyerson JR, Krumpe LR, Constantine B, Wilson J, Buckheit RW, McMahon JB, Subramaniam S, Wlodawer A, O'Keefe BR. 2015. Griffithsin tandemers: flexible and potent lectin inhibitors of the human immunodeficiency virus. Retrovirology 12:6.

27. O'Keefe BR, Vojdani F, Buffa V, Shattock RJ, Montefiori DC, Bakke J, Mirsalis J, D'Andrea A-L, Hume SD, Bratcher B, Saucedo CJ, McMahon JB, Pogue GP, Palmer KE. 2009. Scaleable manufacture of HIV-1 entry inhibitor griffithsin and validation of its safety and efficacy as a topical microbicide component. Proc Natl Acad Sci 106:6099–6104.

28. Gaertner H, Cerini F, Escola J-M, Kuenzi G, Melotti A, Offord R, Rossitto-Borlat I, Nedellec R, Salkowitz J, Gorochov G, Mosier D, Hartley O. 2008. Highly potent, fully recombinant anti-HIV chemokines: reengineering a low-cost microbicide. Proc Natl Acad Sci U S A 105:17706–11.

29. Veazey RS, Ling B, Green LC, Ribka EP, Lifson JD, Piatak M, Lederman MM, Mosier D, Offord R, Hartley O. 2009. Topically applied recombinant chemokine analogues fully protect macaques from vaginal simian-human immunodeficiency virus challenge. J Infect

Dis 199:1525–7.

30. Murphy DJ, Amssoms K, Pille G, Clarke A, Hara MO, Roey J Van, Malcolm RK. 2016. Sustained release of the candidate antiretroviral peptides T-1249 and JNJ54310516-AFP from a rod insert vaginal ring.

31. Gupta P, Lackman-Smith C, Snyder B, Ratner D, Rohan LC, Patton D, Ramratnam B, Cole AM. 2013. Antiviral activity of retrocyclin RC-101, a candidate microbicide against cell-associated HIV-1. AIDS Res Hum Retroviruses 29:391–6.

32. Morrow RJ, Woolfson a. D, Donnelly L, Curran R, Andrews G, Katinger D, Malcolm RK. 2011. Sustained release of proteins from a modified vaginal ring device. Eur J Pharm Biopharm 77:3–10.

33. Veazey RS, Shattock RJ, Pope M, Kirijan JC, Jones J, Hu Q, Ketas T, Marx P a, Klasse PJ, Burton DR, Moore JP. 2003. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. Nat Med 9:343–6.

34. Veselinovic M, Preston Neff C, Mulder LR, Akkina R. 2012. Topical gel formulation of broadly neutralizing anti-HIV-1 monoclonal antibody VRC01 confers protection against HIV-1 vaginal challenge in a humanized mouse model. Virology 432:505–510.

35. Dey B, Lagenaur LA, Lusso P. 2013. Protein-based HIV-1 microbicides. Curr HIV Res 11:576–94.

36. Steinbach JM. 2015. Protein and oligonucleotide delivery systems for vaginal microbicides against viral STIs. Cell Mol Life Sci 72:469–503.

487 37. Gómara MJ, Haro I. 2014. Updating the use of synthetic peptides as inhibitors of HIV-1
488 entry. Curr Med Chem 21:1188–200.
489 38. Whaley KJ, Hanes J, Shattock R, Cone RA, Friend DR. 2010. Novel approaches to vaginal
490 delivery and safety of microbicides: biopharmaceuticals, nanoparticles, and vaccines.
491 Antiviral Res 88 Suppl 1:S55-66.
492 39. Cerini F, Landay A, Gichinga C, Lederman MM, Flyckt R, Starks D, Offord RE, Le Gal F,
493 Hartley O. 2008. Chemokine analogues show suitable stability for development as
494 microbicides. J Acquir Immune Defic Syndr 49:472–6.
495 40. Hartley O, Gaertner H, Wilken J, Thompson D, Fish R, Ramos A, Pastore C, Dufour B,
496 Cerini F, Melotti A, Heveker N, Picard L, Alizon M, Mosier D, Kent S, Offord R. 2004.
497 Medicinal chemistry applied to a synthetic protein: development of highly potent HIV
498 entry inhibitors. Proc Natl Acad Sci U S A 101:16460–16465.
499 41. Lederman MM, Veazey RS, Offord R, Mosier DE, Dufour J, Mefford M, Piatak M, Lifson JD,
500 Salkowitz JR, Rodriguez B, Blauvelt A, Hartley O. 2004. Prevention of vaginal SHIV
501 transmission in rhesus macaques through inhibition of CCR5. Science 306:485–487.
502 42. Gaertner H, Cerini F, Escola JM, Kuenzi G, Melotti A, Offord R, Rossitto-Borlat I, Nedellec
503 R, Salkowitz J, Gorochov G, Mosier D, Hartley O. 2008. Highly potent, fully recombinant
504 anti-HIV chemokines: reengineering a low-cost microbicide. Proc Natl Acad Sci U S A
505 105:17706–17711.
506 43. Cerini F, Gaertner H, Madden K, Tolstorukov I, Brown S, Laukens B, Callewaert N, Harner
507 JC, Oommen AM, Harms JT, Sump AR, Sealock RC, Peterson DJ, Johnson SK, Abramson

SB, Meagher M, Offord R, Hartley O. 2015. A scalable low-cost cGMP process for clinical grade production of the HIV inhibitor 5P12-RANTES in Pichia pastoris. Protein Expr Purif 119:1–10.

44. Lederman MM, Penn-Nicholson A, Cho M, Mosier D. 2006. Biology of CCR5 and its role in HIV infection and treatment. J Am Med Assoc 296:815–826.

45. Wong J, Brugger A, Khare A, Chaubal M, Papadopoulos P, Rabinow B, Kipp J, Ning J. 2008. Suspensions for intravenous (IV) injection: a review of development, preclinical and clinical aspects. Adv Drug Deliv Rev 60:939–54.

46. Curran RM, Donnelly L, Morrow RJ, Fraser C, Andrews G, Cranage M, Malcolm RK, Shattock RJ, Woolfson a D. 2009. Vaginal delivery of the recombinant HIV-1 clade-C trimeric gp140 envelope protein CN54gp140 within novel rheologically structured vehicles elicits specific immune responses. Vaccine 27:6791–8.

47. Senyiğit ZA, Karavana SY, Eraç B, Gürsel O, Limoncu MH, Baloğlu E. 2014. Evaluation of chitosan based vaginal bioadhesive gel formulations for antifungal drugs. Acta Pharm 64:139–56.

48. Karavana SY, Rençbe S, Şenyiğit ZA, Baloğlu E. 2012. A New In-Situ Gel Formulation of Itraconazole for Vaginal Administration. Pharmacol Pharm 3:417–426.

49. Andrews GP, Donnelly L, Jones DS, Curran RM, Morrow RJ, Woolfson AD, Malcolm RK. 2009. Characterization of the rheological, mucoadhesive, and drug release properties of highly structured gel platforms for intravaginal drug delivery. Biomacromolecules 10:2427–2435.

50. Forbes CJ, Lowry D, Geer L, Veazey RS, Shattock RJ, Klasse PJ, Mitchnick M, Goldman L, Doyle L a, Muldoon BCO, Woolfson a D, Moore JP, Malcolm RK. 2011. Non-aqueous silicone elastomer gels as a vaginal microbicide delivery system for the HIV-1 entry inhibitor maraviroc. J Control Release 156:161–9.

51. Yu T, Malcolm K, Woolfson D, Jones DS, Andrews GP. 2011. Vaginal gel drug delivery systems: understanding rheological characteristics and performance. Expert Opin Drug Deliv 8:1309–1322.

52. Wang L, Sassi AB, Patton D, Isaacs C, Moncla BJ, Gupta P, Rohan LC. 2012. Development of a liposome microbicide formulation for vaginal delivery of octylglycerol for HIV prevention. Drug Dev Ind Pharm 38:995–1007.

53. Tien D, Schnaare RL, Kang F, Cohl G, McCormick TJ, Moench TR, Doncel G, Watson K, Buckheit RW, Lewis MG, Schwartz J, Douville K, Romano JW. 2005. In vitro and in vivo characterization of a potential universal placebo designed for use in vaginal microbicide clinical trials. AIDS Res Hum Retroviruses 21:845–53.

54. Grammen C, Ariën KK, Venkatraj M, Joossens J, Van der Veken P, Heeres J, Lewi PJ, Haenen S, Augustyns K, Vanham G, Augustijns P, Brouwers J. 2014. Development and in vitro evaluation of a vaginal microbicide gel formulation for UAMC01398, a novel diaryltriazine NNRTI against HIV-1. Antiviral Res 101:113–21.

55. Owen DH, Peters JJ, Lavine ML, Katz DF. 2003. Effect of temperature and pH on contraceptive gel viscosity. Contraception 67:57–64.

56. Owen DH, Peters JJ, Katz DF. 2001. Comparison of the rheological properties of

Advantage-S and Replens. Contraception 64:393–396.

57. Forbes CJ, Mccoy CF, Murphy DJ, Woolfson AD, Moore JP, Evans A, Shattock RJ, Malcolm RK. 2014. Modified Silicone Elastomer Vaginal Gels for Sustained Release of Antiretroviral HIV Microbicides. J Pharm Sci n/a-n/a.

58. Owen DH, Peters JJ, Katz DF. 2000. Rheological properties of contraceptive gels. Contraception 62:321–6.

59. Barnhart KT, Pretorius ES, Shaunik A, Timbers K, Nasution M, Mauck C. 2005. Vaginal distribution of two volumes of the novel microbicide gel cellulose sulfate (2.5 and 3.5 mL). Contraception 72:65–70.

60. Nel AM, Smythe SC, Habibi S, Kaptur PE, Romano JW. 2010. Pharmacokinetics of 2 dapivirine vaginal microbicide gels and their safety vs. Hydroxyethyl cellulose-based universal placebo gel. J Acquir Immune Defic Syndr 55:161–169.

61. Holt JDS, Cameron D, Dias N, Holding J, Muntendam A, Oostebring F, Dreier P, Rohan L, Nuttall J. 2015. The Sheep as a Model for Preclinical Safety and Pharmacokinetic Evaluations of Candidate Microbicides. Antimicrob Agents Chemother AAC.04954-14.

62. Moss JA, Malone AM, Smith TJ, Kennedy S, Kopin E, Nguyen C, Gilman J, Butkyavichene I, Vincent KL, Motamedi M, Friend DR, Clark MR, Baum MM. 2012. Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. Antimicrob Agents Chemother 56:875–82.

63. Moss JA, Butkyavichene I, Churchman SA, Gunawardana M, Fanter R, Miller CS, Yang F, Easley JT, Marzinke MA, Hendrix CW, Smith TJ, Baum MM. 2016. Combination Pod-

571 Intravaginal Ring Delivers Antiretroviral Agents for HIV Prophylaxis: Pharmacokinetic
572 Evaluation in an Ovine Model. Antimicrob Agents Chemother 60:3759–3766.

573 64. Johnson TJ, Clark MR, Albright TH, Nebeker JS, Tuitupou AL, Clark JT, Fabian J, McCabe
574 RT, Chandra N, Doncel GF, Friend DR, Kiser PF. 2012. A 90-day tenofovir reservoir
575 intravaginal ring for mucosal HIV prophylaxis. Antimicrob Agents Chemother 56:6272–83.

576 65. Richardson JL, Illum L, Thomas NW. 1992. Vaginal Absorption of Insulin in the Rat: Effect
577 of Penetration Enhancers on Insulin Uptake and Mucosal Histology. Pharm Res An Off J
578 Am Assoc Pharm Sci 9:878–883.

579 66. Rosenzweig M, Walzer M. 1943. Absorption of protein from the vagina and uterine
580 cervix. Am J Obstet Gynecol 45:286–290.

581 67. Okada H, Yamazaki I, Ogawa Y, Hirai S, Yashiki T, Mima H. 1982. Vaginal Absorption of a
582 Potent Luteinizing Hormone-Releasing Hormone Analog (Leuprolide) in Rats I: Absorption
583 by Various Routes and Absorption Enhancement. J Pharm Sci 71:1367–1371.

584 68. McKay PF, Mann JFS, Pattani A, Kett V, Aldon Y, King D, Malcolm RK, Shattock RJ. 2017.
585 Intravaginal immunisation using a novel antigen-releasing ring device elicits robust
586 vaccine antigen-specific systemic and mucosal humoral immune responses. J Control
587 Release 249:74–83.

588 69. Kimball AB, Javorsky E, Ron ES, Crowley, W, Langer R. 2016. A novel approach to
589 administration of peptides in women: Systemic absorption of a GnRH agonist via
590 transvaginal ring delivery system. J Control Release 233:19–28.

591 70. Nel AM, Coplan P, Smythe SC, McCord K, Mitchnick M, Kaptur PE, Romano J. 2010.

592 Pharmacokinetic assessment of dapivirine vaginal microbicide gel in healthy, HIV-
593 negative women. AIDS Res Hum Retroviruses 26:1181–90.

594 71. Hendrix CW, Chen BA, Guddera V, Hoesley C, Justman J, Nakabiito C, Salata R, Soto-
595 Torres L, Patterson K, Minnis AM, Gandham S, Gomez K, Richardson B a, Bumpus NN.
596 2013. MTN-001: Randomized Pharmacokinetic Cross-Over Study Comparing Tenofovir
597 Vaginal Gel and Oral Tablets in Vaginal Tissue and Other Compartments. PLoS One
598 8:e55013.

599 72. Karim SSA, Kashuba ADM, Werner L, Karim QA. 2011. Drug concentrations after topical
600 and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in
601 women. Lancet 378:279–81.

602 73. Malcolm RK, Lowry D, Boyd P, Geer L, Veazey RS, Goldman L, Klasse PJ, Shattock RJ,
603 Moore JP. 2014. Pharmacokinetics of a CCR5 inhibitor in rhesus macaques following
604 vaginal , rectal and oral application 1325–1329.

605 74. Cerini F, Offord R, McGowan I, Hartley O. 2017. Stability of 5P12-RANTES, A Candidate
606 Rectal Microbicide, in Human Rectal Lavage. AIDS Res Hum Retroviruses aid.2016.0199.

607 75. Owen DH, Katz DF. 1999. A vaginal fluid simulant. Contraception 59:91–5.

608 76. Wang NX, Sieg SF, Lederman MM, Offord RE, Hartley O, Von Recum H a. 2013. Using
609 glycosaminoglycan/chemokine interactions for the long-term delivery of 5P12-RANTES in
610 HIV prevention. Mol Pharm 10:3564–3573.

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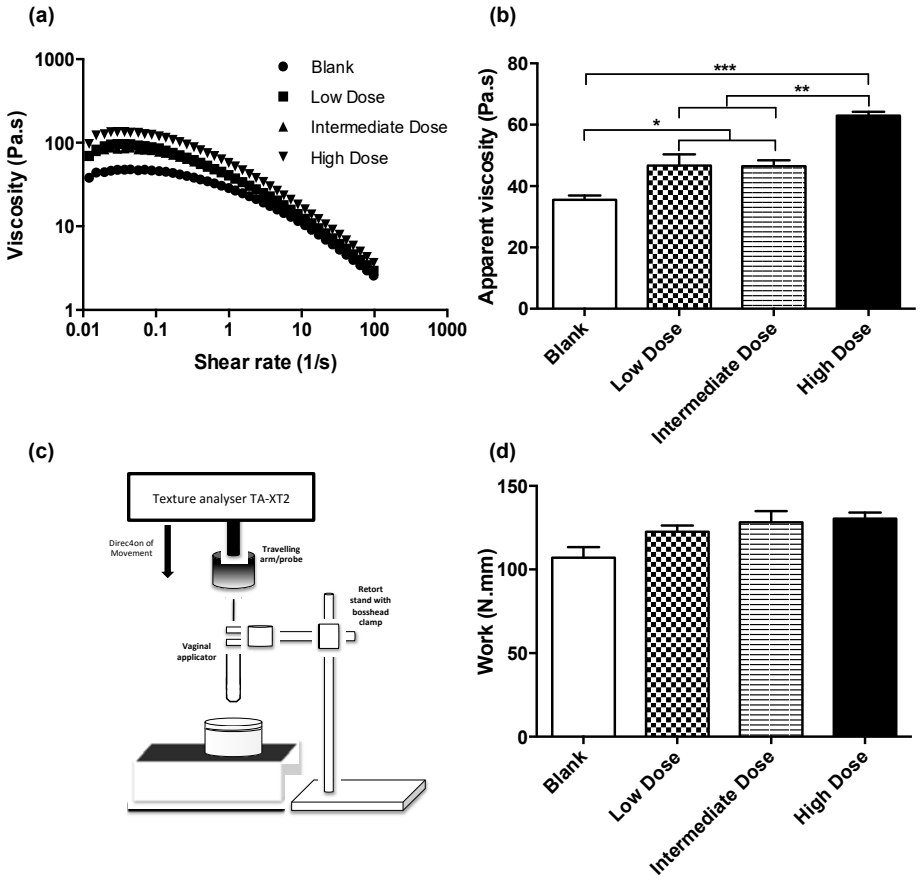

614 **Figure 1.** Characterisation of hydroxyethylcellulose (HEC) gels containing low dose: 1.24 mg/ml
615 5P12-RANTES; intermediate dose: 6.18 mg/ml 5P12-RANTES; high dose: 32 mg/ml 5P12-RANTES.
616 **(a)** Viscosity of gel formulations determined by continuous flow rheology and **(b)** through
617 application of the Power Law was to determine apparent viscosity. Results displayed as mean +
618 SEM, *n*=3. Significance determined by one-way ANOVA post hoc pairwise Tukey's test, **P*<0.05,

619 **P<0.005, ***P<0.0005. **(c)** Setup for syringeability testing using a texture profile analyser (TA-
620 XT2) and a vaginal applicator filled with gel which was employed to determine the **(d)** work
621 required (N.mm) to expel 4 g of gel, n=3, mean + SEM.

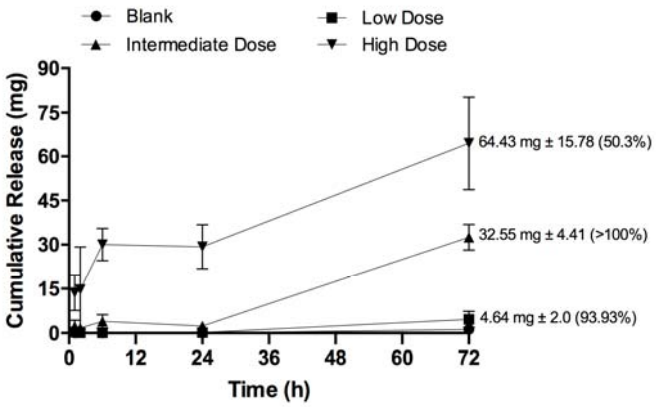

Figure 2. Cumulative release of 5P12-RANTES from hydroxyethylcellulose (HEC) gels containing a low (1.24 mg/g), intermediate (6.18 mg/g) or high (32.0 mg/g) dose of the CCR5 inhibiting peptide, as detected by ELISA. Gels were incubated in simulated vaginal fluid, 37°C, throughout the study, *n*=3, mean ± SEM.

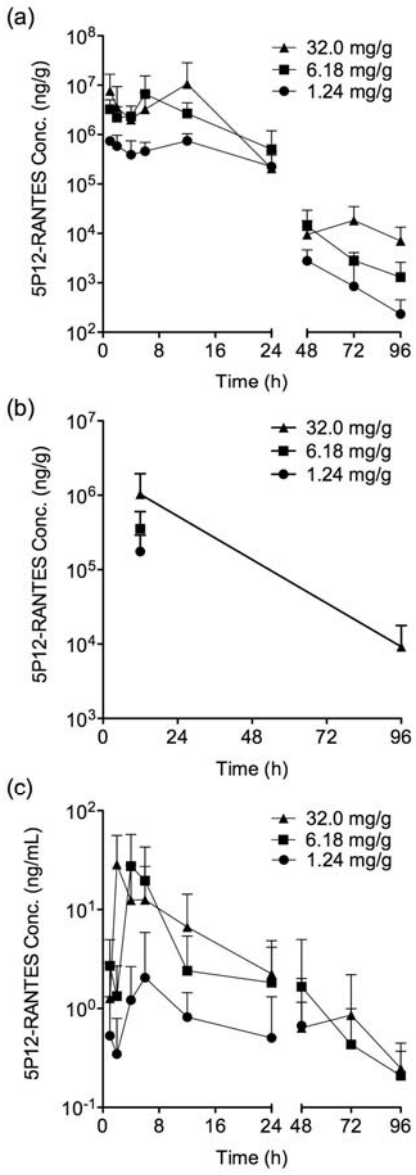

Figure 3. Pharmacokinetic concentrations of 5P12-RANTES in (a) vaginal fluid, (b) vaginal tissue, and (c) serum of sheep following single dose vaginal administration of hydroxyethylcellulose (HEC) gels containing low (1.24 mg/g), intermediate (6.18 mg/g) and high (32.0 mg/mL) concentrations of 5P12-RANTES. Plot symbols represent mean + standard deviation of four replicates.

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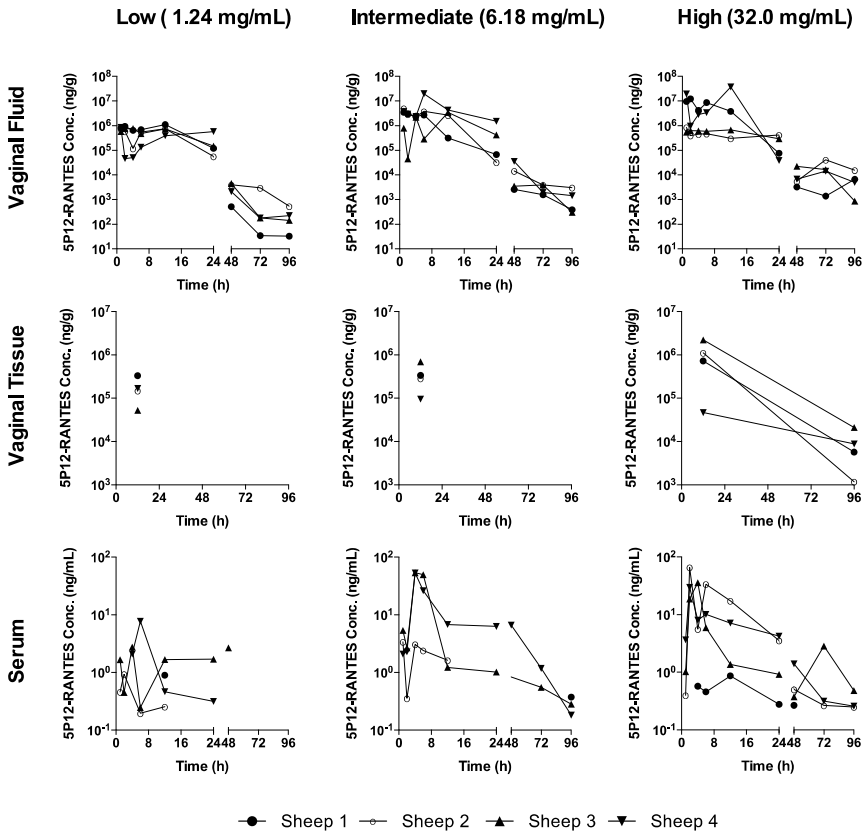

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629 **Figure 4.** 5P12-RANTES pharmacokinetic profiles for individual sheep following single dose
630 vaginal administration of hydroxyethylcellulose (HEC) gels containing low (1.24 mg/g),
631 intermediate (6.18 mg/g) and high (32.0 mg/mL) concentrations of 5P12-RANTES. Plot symbols
632 represent mean + standard deviation of four replicates.

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633 **Table 1.** Description of 5P12-RANTES gel formulations and the concentrations measured in sheep
634 vaginal fluid and serum vaginal following single dose vaginal gel administration. C_{max} (maximum
635 serum concentration) and *AUC* (area under the curve) data are presented as means ± standard
636 deviations of four replicates; t_{max} data (time to reach C_{max}) are presented as median and range of
637 four replicates.
638

5P12-RANTES conc. in gel (total 5P12-RANTES dose administered)	*Vaginal fluid*			*Serum*		
	C_{max} (µg/g)	t_{max} (h)	$AUC_{0\text{-}last}$ (µg·h/g)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}last}$ (ng·h/mL)
1.24 mg/g (4.94 mg)	869 ± 167	8.0, 1–12	15100 ± 2600	3.09 ± 3.23	5.0, 2–12	46.4 ± 52.0
6.18 mg/g (24.7 mg)	7920 ± 7890	3.5, 1–12	71300 ± 58900	27.6 ± 29.7	4.0, 1–96	243 ± 279
32.0 mg/g (128 mg)	12800 ± 17300	7.0, 1–12	130000 ± 172000	33.0 ± 26.6	3.0, 2–12	258 ± 192